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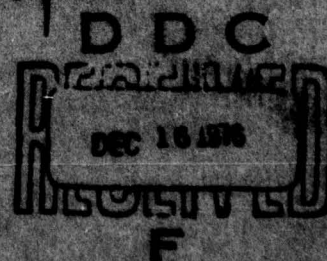
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ABSTRACT

The present *in vitro* study was conducted to explore influences modifying glucose uptake in canine blood administered an estimated LD_{100} *E. coli* endotoxin. Particular emphasis was given to assay the role that leukocytes perform in glucose utilization. Results show significant increases in glucose uptake and lactic acid production 1-3 hours following endotoxin addition. Accelerated glucose uptake greatly exceeded basal values and was attributed to increased activity of the white cell. The excess glucose required by the white cell was independent of temperature between the ranges of $34°-41°C$. Endotoxin, however, simultaneously exerted adverse effects by depressing glucose metabolism below predicted values on the basis of Q_{10} findings and increased white cell mortality rate. Blood pretreated with sublethal doses of endotoxin *in vivo* was studied under *in vitro* conditions. All endotoxin-pretreated animals survived superlethal doses of endotoxin. Pretreated blood studied *in vitro* exhibited accelerated hypoglycemia when subjected to endotoxin, "activation" of the blood elicited by increasing numbers of white blood cells rather than by enhancement of individual cell activity.

INDEX TERMS: endotoxin, leukocytes, glucose metabolism, blood and endotoxin, canine endotoxin shock, *in vitro* endotoxin, Q_{10}

INTRODUCTION

The seriousness of septic shock has been emphasized in a recent report estimating 132,000 deaths a year from a total number of 330,000 cases (22). The incidence of septic shock has increased almost twenty-fold over the past 20 years (22), and therapeutic procedures have not been generally successful (4). Special efforts have therefore been made in recent years to determine its underlying mechanisms in order to develop a successful therapy.

Documented pathophysiological mainfestations in canine endotoxin shock include progressively developing hypoglycemia correlated with systemic hypotension, hepatosplanchnic pathology, and death (21). It has been proposed that hypoglycemia results from the adverse effects of impaired liver function on gluconeogenesis (4,9,16,17,23) and from increased glucose utilization (1,26), including an accelerated glucose uptake by the blood (1,21). Administration of 50% glucose was found to significantly benefit endotoxin-shocked animals (20,21,26). These recent findings suggest that the degree of lethality may be directly related to the extent of the discrepancy between the production of glucose and its utilization during shock.

The present studies were designed to explore factors influential in modifying the uptake of glucose by the blood itself following administration of endotoxin. Findings reveal both accelerative and depressant factors simultaneously set into motion by endotoxin which may directly involve leukocyte participation and significantly influence the lethal outcome of shock.

METHODS

Experiments were designed to assay factors influencing the metabolism of certain blood constituents, especially the white blood cells, in the presence of endotoxin. Studies were carried out on blood samples from 30 donor dogs.

Adult mongrels of random sex, selected for robust health and absence of heart worms, were treated for intestinal parasites and conditioned in the animal facility for 3 to 6 weeks prior to _in vivo_ and _in vitro_ experiments. Studies were divided into paired control and experimental groups in which responses to _E. coli_ endotoxin (Difco, Detroit) or saline could be recorded and compared.

An _in vitro_ system served as the primary model because of its demonstrated usefulness (21) and simplicity of experimental design, and because the disappearance rate of glucose after endotoxin closely compares to the rate of development of hypoglycemia after endotoxin in the dog deprived of the organs of gluconeogenesis (1,26). Blood for _in vitro_ studies was drawn intravenously from unanesthetized, non-fasted donor animals with plastic syringes wet with heparin (1,000 U/ml concentration). Blood samples were divided into 10 ml volumes in separate plastic test tubes to each of which was added 0.1 ml heparin (20,000 U/ml), followed by incubation in a water bath at predetermined controlled temperatures for a 3-7 hour observation period. Endotoxin was added to the experimental tube of each group at a calculated LD$_{100}$ concentration (0.025-0.035 mg/ml)(21), while the control tube of blood from the same animal was administered an equal volume of saline. Blood was centrifuged in some experiments, and red cells, buffy coat, and plasma were separated. Red cells were then washed with a glucose-saline solution and resuspended in saline with normal glucose concentrations. Parameters monitored included red and white cell counts (Coulter Counter); blood gases and pH (Instrumentation Laboratories Analyzer); glucose (Beckman Glucose Analyzer, accuracy of ±3 mg%); and lactate (Hohorst, H. J., _Methods of Enzymatic Analysis_, H. V. Bergmeyer, Ed., Academic Press, New York, 1965)..

Experiments were designed to assay the role of the blood in the hypoglycemic response to LD$_{100}$ endotoxin by measuring the rate of glucose disappearance _in vitro_ in blood receiving endotoxin or saline separately. _In vitro_ studies

defined two conditions of glucose uptake: (a) basal metabolic requirements and (b) "excess" glucose requirement for white cell activity due to endotoxin. In vitro blood samples receiving saline of equal volume as that of endotoxin served as indicators for basal metabolic processes. The quantity of glucose disappearing in the control series was subtracted from that disappearing in the endotoxin group, providing an estimate of excess glucose utilized as a result of the action of endotoxin.

In the first series of experiments, metabolic responses of normal canine blood were studied including glucose uptake and lactic acid production. Factors modified during the experiments were temperature and blood constituents including red blood cells, plasma, and buffy coat. Possible detrimental effects on white blood cell function and longevity were assayed. In a second series of experiments, blood was obtained from animals previously receiving sublethal injections of endotoxin and studied in vitro. These studies were conducted to determine if certain blood constituents, particularly the white cells, were "activated" by prior sublethal endotoxin exposure. Statistics were carried out including paired and unpaired data analysis utilizing the t test. Twenty-two in vitro experiments on effects of temperature, 21 experiments on metabolic responses, 12 activation studies, and 12 animal in vivo experiments were included in the study.

RESULTS

The first series of in vitro experiments was carried out to examine metabolic changes in whole heparinized canine blood following addition of Table 1 LD100 E. coli endotoxin. Results from 18 experiments in Table 1 show that endotoxin elicits a significant increase in glucose uptake by the blood, in comparison to the control series ($p < 0.005$) receiving saline instead of endotoxin during a 3-hour period. Concomitantly, there is a

notable increase in lactic acid and decrease in pH, as compared to the saline control group ($p \leq 0.01$). Changes in glucose and lactic acid produced by administration of endotoxin could not be altered by addition of insulin (Table 1) when the existing blood concentration of insulin was raised by 0.7 U/ml. These findings suggested that glycolytic activity is elevated above the basal metabolic level of the control blood samples when endotoxin is added, although the responsible blood components are not identified.

Studies were then designed to identify the specific blood components responsible for the accelerated uptake of glucose after endotoxin. The upper

Table 2

portion of Table 2 illustrates the in vitro response to endotoxin by washed red blood cells suspended in a saline-glucose (approximately 100 mg%) solution. Results show that endotoxin does not cause an increased disappearance of glucose above that of the control administered saline alone with no endotoxin. Table 2 illustrates the effects of incorporating plasma or buffy coat into the washed red blood cell (RBC) suspension following addition of endotoxin, after establishment of base-line values. Addition of plasma to the RBC suspension produced no change over that of the RBC suspension alone. The combination of RBC's, buffy coat in small volume of plasma, and endotoxin elicited notable increases in glucose disappearance as indicated by the rapidly declining values of glucose concentration in the test tubes. These findings indicated that the primary cellular components essential for accelerated glucose disappearance after endotoxin are in the buffy coat. To evaluate the possible role of the white blood cell as a component of the buffy coat responsible for the elevated glucose uptake after endotoxin, an in vitro experiment was conducted to manipulate white blood cell (WBC) concentrations. Varying amounts of buffy coat (approximately 0.5 ml) were added to the blood prior to addition of LD100 endotoxin. Glucose uptake increased rapidly after endotoxin as white blood

cell concentration increased in the range of 11,900 to 34,500 WBC/mm^3. These observations implicated the WBC as being responsible for the increased uptake of glucose after endotoxin administration in vitro.

Preliminary observations suggested that elevated temperature enhanced the disappearance rate of glucose after addition of endotoxin in vitro, and since experiments described below document a profound febrile response to endotoxin in vivo, the effects of temperature and endotoxin on glucose uptake and metabolic rate in vitro were determined. Table 3 arrays the results from 32 in vitro experiments carried out at 34°C and 41°C and illustrates changes in glucose concentrations for a 1-hour incubation period under four conditions: temperatures of 34°C and 41°C and endotoxin vs. saline additions to in vitro blood. Findings document an increased uptake of glucose as a function of elevated incubation temperatures in both the saline controls (p=0.001) and endotoxin series (p=0.001), thus supporting an important metabolic-accelerating effect of elevated blood temperature on the cellular components of the blood. It was also noted that glucose uptake is significantly higher when endotoxin is added to the blood vs. saline alone at each temperature (p=0.005 at 34°C and p=0.02 at 41°C). Additional data on metabolic rate were obtained in order to relate the magnitude of the increases in metabolic rate between the endotoxin vs. saline series. Q10 calculations were carried out for both control and experimental groups. Mean Q10 values were 2.78 in the saline series and 2.12 after endotoxin, which were significantly different (p=0.05). Data from Table 3 illustrate independent effects of blood temperature and endotoxin: an accelerating action on glucose uptake, elicited separately by elevated temperature and endotoxin, and a concomitant depressant action on metabolism by endotoxin, as revealed by the smaller increase in metabolic rate (Q$_{10}$) in the endotoxin series as compared to the saline control group.

Table 3

Findings from the present study suggest that the white blood cell is the primary component of the blood responsible for the accelerated uptake of glucose following endotoxin addition. Studies were conducted to quantitate glucose consumption per WBC, including the added influence of altered tempera-

Table 4   ture. Table 4 displays the excess glucose uptake on the basis of a single white cell. Data were obtained by subtracting the glucose uptake in the control (saline) series from the endotoxin group. This was done to remove the quantity of glucose consumed by cellular components of the blood in the resting state, in order that the additional glucose drain required for phagocytosis or additional cell activity may be quantitated. Three sets of experiments at varying temperatures were carried out with blood in vitro to which LD_{100} endotoxin was added. Data were obtained for the period 0-60 minutes post-endotoxin, and consideration was given to the loss of WBC's during this period (Table 3) in calculating excess glucose consumption per cell at the end of one hour of incubation. Glucose uptakes at 34°, 38°, and 41°C were 6.8×10^{-9}, 9.5×10^{-9}, and 6.7×10^{-9} mg/WBC/hr, respectively, and indicate relatively constant quantities of glucose regardless of the existing incubation temperature. Data suggest that the excess glucose used after endotoxin administration does not vary as a function of temperature, although total energy requirements (resting plus active state) increase with temperature elevation (Table 4).

Preliminary observations suggested that white blood cells may be destroyed by endotoxin, and in vitro experiments were carried out to assay this possibility.

Table 5   Results from 22 separate in vitro experiments are shown in Table 5 which illustrate the effects of endotoxin on WBC survival in a 4-hour period. Incubation temperatures were selected in order to include both hypothermic and hyperthermic phases of shock. Results show that WBC concentrations significantly decrease

after endotoxin in blood incubated for 3 hours at 38°C (p=0.01) and by 220 minutes at 41°C (p=0.02) in comparison to the control, saline-administered series. Since the rate of cell destruction was appreciably less in blood to which saline alone was added (Table 5), a WBC-destroying action of LD_{100} endotoxin is established.

Two mechanisms responsible for accelerated uptake of glucose in blood after endotoxin have been identified in the present study: one is the energy cost for increased white cell activity and the other is elevated total metabolic demand because of increased temperature. An additional possible added energy drain on existing metabolic requirements to be explored was a possible activated state of white blood cells. Experiments were designed to study the rate of glucose disappearance brought about with experimentally modified WBC activity. Twelve unanesthetized animals were studied during a 4-day period: six "activated" animals receiving daily sublethal intravenous injections of endotoxin and six "non-activated" animals receiving saline injections for a 3-day period. On the fourth day, but before endotoxin or saline administration, venous blood was removed from both animals, heparinized, transferred to the in vitro system, and studied as described above. Endotoxin (LD_{100}) was administered to both Figure 1 activated and non-activated blood in vitro (Figure 1). Results from six paired experiments demonstrate a rapid acceleration of glucose disappearance from activated blood (endotoxin pretreated) in both control and experimental blood samples studied in vitro. Blood glucose concentrations fell more gradually in non-activated blood in a separate series of six experiments (Figure 1). Blood glucose concentrations at 60 minutes in activated blood were significantly lower than the non-activated blood when compared between both saline control (p=0.005) and endotoxin-treated (p=0.001) groups. Glucose uptake in the acti-

vated blood given endotoxin was greater than its saline control at 120 minutes
(p=0.05).

An analysis of data shown in Table 6 from the same studies as depicted
in Figure 1 indicate that glucose uptake per WBC is not accelerated in the
activated state (pretreatment with endotoxin) above that of the non-activated
group (saline pretreatment only). Subtracting glucose uptake in the in vitro
saline experiments from that in the endotoxin (LD_{100}) group provides the esti-
mate of glucose requirements for increased WBC activity: in the activated group,
4.3×10^{-9} mg glucose/WBC/hr was used while in the non-activated series the
mean value was 7.2×10^{-9} mg/WBC/hr, no significant difference being observed
between the two (p>0.05). The WBC concentrations in the activated series
averaged $40,400/mm^3$ while those from the non-activated group averaged 11,700
WBC/mm^3 at 60 minutes post-endotoxin, and these were significantly different
from each other (p=0.02).

Parallel responses of the unanesthetized dogs which provided blood for
the in vitro studies described in Figure 1 and Table 6, which simultaneously
received $2 \times LD_{100}$ intravenous endotoxin injections on the fourth day, are
shown in Figures 2A, 2B, and 2C. Mean changes in blood glucose, WBC concen-
tration, and body temperature in six activated and six non-activated dogs are
presented in paired experiments. All animals in the activated group (N=6)
survived a $2 \times LD_{100}$ endotoxin administration (30-day survival period), while
all dogs in the non-activated group (N=6) died in an average survival time of

Figure 2A 7 hours. Figure 2A arrays mean blood glucose concentrations in six activated
and six non-activated unanesthetized dogs administered $2 \times LD_{100}$ endotoxin
following base-line control values. Mean glucose concentration fell from
approximately 90 mg% at zero time to about 55 mg% for the first 2 hours in the

activated group (p<0.005), while it rose from 90 mg% to approximately 120 mg%
in the non-activated group (p>0.05), where it remained at that level for 2 hours.
The 2-hour glucose values were significantly different between the two groups
(p=0.005). Glucose concentrations steadily rose in the activated dogs from the
low value of 50 mg% at 2 hours to the control value of 90 mg% in 8.5 hours
(p<0.001), while glucose concentrations progressively declined in the non-
activated group from about 120 mg% at 2 hours to a mean of 55 mg% at 7 hours
(p>0.05) in two survivors of the six non-activated animals. Figure 2B provides
Figure 2B mean changes in WBC concentrations in activated and non-activated dogs given
2 X LD_{100} endotoxin. Initial mean base-line control WBC concentrations were
approximately 43,000 and 13,000/mm^3 in activated and non-activated dogs,
respectively. One hour following endotoxin, marked leukopenia was observed
at its greatest extent in both groups (approximately 5,000 counts/mm^3). From
one hour to 8.5 hours, WBC concentrations progressively rose to near control
values in the activated group and to above the mean control value in the non-
Figure 2C activated series. Figure 2C presents mean changes in body temperature in
activated and non-activated dogs receiving 2 X LD_{100} endotoxin. Febrile
responses are seen in both groups, with temperatures approximately equal at
3 hours post-endotoxin (40°C), both elevated from starting temperatures of
39°C (p<0.001) and 38°C (p<0.005) in activated and non-activated animals,
respectively. From 3 to 7 hours temperatures remained elevated in the non-
activated group (above 40°C) and progressively fell in the activated series to
the control value of 39°C. Temperatures in the activated group were signifi-
cantly lower than the non-activated series at 5.5 hours (p=0.005) and 7 hours
(p=0.02).

DISCUSSION

Progressively developing hypoglycemia in dogs administered endotoxin has been recently documented and found to be associated with systemic hypotension, hepatosplanchnic pathology, and death (20). The cause of hypoglycemia has been the subject of much recent research in shock. Impaired glucose production as a result of depressed hepatic function has been suggested as a primary factor in the development of hypoglycemia because of adverse effects on gluconeogenesis (4,9,16,17,23). It has not been possible to clearly implicate increased glucose uptake in endotoxin shock as a major factor accounting for hypoglycemia except for recent reports indicating an accelerated uptake of glucose by the blood itself (1,21).

The first aim of the study was to identify the particular component of the blood primarily responsible for the previously reported accelerated disappearance of glucose in the blood after endotoxin (1,21). Findings suggested that the red blood cell alone, or in combination with plasma, was not responsible for the increased uptake of glucose after endotoxin. Buffy coat added to red cell and plasma resulted in a notable acceleration of glucose uptake when endotoxin was added. Further, increasing white cell concentration by adding buffy coat elevated glucose uptake in direct proportion to white cell count. The possibility that red cell metabolism may be accelerated in the presence of white cells and endotoxin was not evaluated.

Assumptions made in the present study are that glucose is the primary fuel utilized and that the white blood cell is the chief unit present in the buffy coat, which demonstrates acceleration of cell activity following endotoxin administration in vitro. In substantiation of these assumptions, it has been pointed out that glucose is necessary for the supply of energy required during phagocytosis (7), and that endotoxin injection in rabbits results in an increased

utilization of glucose by circulating leukocytes (8). In addition, circulating neutrophils have been identified as the key factors in the clearance of bacterial organisms from the blood of dogs (27); leukocytes detoxify endotoxin in vitro in rabbit serum (28); and blood from endotoxin-treated rabbits possesses a significant phagocytic capacity (24). The addition of endotoxin to human blood stimulates its metabolism, which was concluded to be due to increased respiratory activity of the leukocytes (29). Finally, Braude's group traced the early disappearance of radioactively-labelled endotoxin into the buffy coat of rabbits, where it then passed into the liver during the leukopenic stage (3).

Data from the present study suggest that the white blood cell is the primary blood constituent responsible for the excess glucose uptake exceeding basal values following addition of endotoxin in vitro. Further, the marked increase in lactic acid suggests utilization of the glycolytic pathway in phagocytosis as described by others (7,8). The increase in glucose uptake over basal values in the in vitro studies is probably due to accelerated activity of the white cells in response to endotoxin exposure. The additional "excess" glucose required in this instance may result from increased phagocytic activity of the white cell although other possible energy-requiring functions of the various particulate constituents of the blood may have been stimulated by endotoxin.

The unanesthetized dogs of the present study administered a lethal dose of endotoxin demonstrated increases in body temperature averaging 2.5°C. This rise in temperature, occurring during the early phase of shock when glucose disappearance takes place at an accelerating pace in vitro, suggests that fever may add an additional drain to the available glucose. Therefore, the effects of elevated blood temperature on the rate of glucose disappearance in vitro after endotoxin were studied to determine the influence of accelerated metabolic

need on glucose uptake. Results show that uptake for basal requirements was significantly increased by temperature elevation with or without endotoxin; however, the excess quantity of glucose required only after endotoxin addition was independent of temperature. This observation suggests that a constant level of white cell phagocytic energy processes may occur within a wide range of temperature, while basal metabolic requirements obey the expected Q_{10} relationship. Q_{10} values after LD_{100} endotoxin in vitro averaged about 2.1, and in the saline controls approximately 2.8 during the first hour. A substantial quantity of additional glucose would therefore be required for basal metabolic purposes during the febrile phase of shock, particularly when one takes into account the probable increased glucose demand by sessile cellular components of the RE system. This increased fuel requirement might exert a significant drain on the available glucose stores, particularly in the presence of severely depressed hepato-splanchnic blood flow and the ensuing liver dysfunction in canine endotoxin shock (4,9,16,20).

Although temperature elevation increased white blood cell metabolic rate, the introduction of endotoxin limited the extent of increase. During the first hour of exposure to endotoxin, there was an approximate 40% depression of total white cell metabolism in contrast to the saline controls, as estimated from the difference in Q_{10} findings ($\frac{1.8 - 1.1}{1.8}$ X 100). This apparent depression of function would be present during the leukopenic phase of shock, possibly contributing to the pathogenesis of hepatic dysfunction (4,9,16,20). Endotoxin added to blood in vitro resulted in the death of approximately 10% of the WBC, a rate significantly greater than the saline controls. However, this rate appears less than that in vivo due to the absence of cell migration. Also, the time course of in vitro studies (3-4 hours) limited the time of cell exposure to endotoxin in contrast to the in vivo state. Other limitations of the in vitro system were

that tissue sources for the release of toxic factors and adrenergic agents were excluded, thus sparing the white cells from possible adverse factors contributing to their premature death.

An additional energy drain on the existing metabolic requirements of white blood cells in shock might be due to a change in the state of "activation" of the cell. Experiments conducted in the present study to modify white cell metabolic activity revealed that sublethal injections of endotoxin given daily to unanesthetized dogs elicited rapid leukopenia and fever, followed by progressively developing leukocytosis. On the day of challenge, lethal doses of endotoxin were simultaneously administered in vivo and in vitro. Results demonstrate a markedly accelerated uptake of glucose in vitro both in the presence and absence of endotoxin, with the greatest glucose uptake occurring after endotoxin. The data suggest that "activation" of the white blood cells as a result of sublethal endotoxin pretreatment is elicited on the basis of increased numbers of cells, rather than increased metabolic activity per cell. Of interest were the protective aspects of endotoxin pretreatment: activation of blood protected the animals completely from the lethal action of endotoxin, even at superlethal levels. Activated blood subjected to LD_{100} endotoxin in vitro demonstrated a markedly accelerated uptake of glucose on the basis of total glucose utilized. However, "excess" glucose (above basal) expressed per white cell was found to be a constant quantity in both activated and non-activated blood, thus suggesting that sublethal pretreatment with endotoxin may protect against a lethal insult by mobilizing greater numbers of white cells. While blood glucose concentrations fell extremely rapidly in vitro after LD_{100} endotoxin in the endotoxin-pretreated blood, glucose levels in vivo declined gradually during a similar period in intact animals pretreated with endotoxin

following 2 X LD$_{100}$ endotoxin and then progressively returned to normal. In contrast, control animals not pretreated with endotoxin demonstrated hyperglycemia for the first 2 hours post-endotoxin followed by progressively developing hypoglycemia and death. All animals pretreated with endotoxin survived for 30 days and all not so treated died within a mean time of 7 hours. Endotoxin pretreated animals had minimal diarrhea and within 9 hours after endotoxin were eating and drinking and were mentally alert. White cell concentrations were significantly elevated in the endotoxin-pretreated group; since animals were notably protected against the effects of massive endotoxin challenge, there is a possibility that the greater number of circulating white cells effected rapid removal of circulating endotoxin. Leukocytes have been reported to exert a significant role in phagocytosis, although serum and opsonins may also perform key roles (28,30). Increased resistance to endotoxin occurs on the basis of an increased functional capacity of the RES to remove bacterial toxin from the blood, and protection is not related to antibody formation (2). Tolerance to endotoxin involves increased RES activity as well as other factors (5,6,19,25) and is not passively transferred with serum from resistant animals (6). In one study, endotoxin detoxifying activity was not found in cell elements of the circulating blood (11), although species differences may have explained these observations. It has been proposed that it is not really an increased RES activity which produces tolerance to endotoxin but a greater stability of carbohydrate metabolism in the liver (13). The accompanying febrile response may perform a beneficial role in enhancing metabolic activities of the white cell or improving hepatic hemodynamics through the release of a pyrogen derived from the leukocyte (14) or endotoxin itself (12). Braude and others have traced the passage of radioactively-labelled endotoxin from the plasma, but not the

red cell, to the buffy coat and finally to the liver during the early clearance period associated with leukopenia, fever, and diarrhea (3). It seems likely that if phagocytosis in the buffy coat were sufficiently effective, the liver might be spared the added stress of endotoxin detoxification (9) and possible depression of function as a direct action of endotoxin (17); and as a result the maintenance of normal gluconeogenic capabilities (13,16) would be preserved during the subsequent period of shock.

This study suggested the protective influences of leukocytosis stimulated by sublethal injections of endotoxin. Since survivability seemed to be so strongly associated with leukocytosis, there are possible therapeutic implications regarding the role of the white blood cell in endotoxin shock. Recent reports have described beneficial effects of transfused white blood cells as a treatment for septicemia in neutropenic patients (15) and as a therapy for Pseudomonas septicemia in neutropenic dogs (10). Increased survival has been documented in patients thus treated and the numbers of bacteria in septic animals were greatly diminished following white cell transfusion therapy. Administration of hypertonic glucose has resulted in an increased clearance of E. coli from the blood of dogs (18), suggesting that supplying substantial metabolic support for accelerated leukocyte activity may enhance survival in septic shock.

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TABLE 1. Effect of E. coli endotoxin (LD100) on glucose uptake, lactic acid production and pH in vitro[a] in blood

| | | Initial WBC Count | Time (minutes) | | | | | | | | |
| | | | Glucose (mg/100 ml)[b] | | | | Lactic Acid[b] (mg/100 ml) | | pH | | |
			Control	+60	+120	+180	Control	+180	Control	+60	+180
Control Group (Saline Only) (N=6)	Mean	15,300	83	69[c]	53	40	13.0	34.0	7.40	7.37	7.31
	SE	1,700	2.5	2.3	2.9	2.4	1.9	3.5	0.01	0.01	0.01
	P			0.001	0.001	0.001		0.001		0.02	0.01
Experimental Group (Endotoxin) (N=6)	Mean	15,300	83	55[c]	35	19	13.0	43.4	7.40	7.33	7.25
	SE	1,700	2.5	4.2	5.4	3.4	1.9	4.5	0.01	0.02	0.02
	P			0.001	0.001	0.001		0.001		0.005	0.005
	P[d]			0.005	0.005	0.001		0.05		0.01	0.01
Experimental Group plus Insulin (Endotoxin Insulin) (N=6)	Mean	15,300	83	55[c]	35	20	13.0	42.3	7.40	7.33	7.25
	SE	1,700	2.5	4.0	5.4	3.3	1.9	4.4	0.01	0.02	0.02
	P			0.001	0.001	0.001		0.005		0.005	0.001
	P[e]			0.001	0.005	0.001		NS		0.02	0.02

[a]Endotoxin (0.035 mg/ml) or comparable volume of saline (0.035 ml/ml) added after control values. [b]Absolute concentrations of glucose and lactate are shown at indicated times. [c]Insulin (0.7 U/ml) or comparable volume of saline (0.007 ml/ml) added after +60 minute values. [d]Endotoxin group compared to saline group (p value). [e]Endotoxin + insulin group compared to saline group (p value). (Symbol "p" = significance at 60, 120, and 180 minutes compared to zero time values.)

TABLE 2. Effect of blood constituents on glucose uptake after E. coli endotoxin (LD$_{100}$) in vitro

Glucose uptake by red blood cells after endotoxin

| | Time (min) | Glucose (mg/100 ml)[a] | |
		RBC[b] + Saline (No Endotoxin)	RBC + Endotoxin
Expt. 1	0	91	91
	+60	70	69
	+120	60	61
	+180	54	56
	+360	40	40

Glucose uptake by red blood cells in combination with plasma or buffy coat after endotoxin

Time (min)	Glucose (mg/100 ml)[a]		
	RBC + Endotoxin	RBC + Plasma + Endotoxin	RBC + Buffy Coat + Endotoxin
Expt. 2 Pre-Endotoxin			
0	93	93	93
Post-Endotoxin			
+120	62	62	61
	[c]	[d]	[e]
+180	47	60	41
+240	42	52	9
+360	36	44	1
Expt. 3 Pre-Endotoxin			
0	78	78	78
	[c]	[d]	[e]
Post-Endotoxin			
+60	58	56	21
+120	43	41	11
+180	29	30	11
+300	20	16	6

[a]Absolute concentrations of glucose are shown at indicated times. [b]Washed red blood cells, suspended in saline and reconstituted with glucose. [c]Saline added. [d]Plasma added. [e]Buffy coat added (with minimal plasma).

TABLE 3. Effects of temperature and E. coli endotoxin on glucose uptake and metabolic rate in blood (Blood from each of 8 dogs simultaneously studied *in vitro* at 37°C and 41°C with addition of saline or LD100 endotoxin)

	Saline (Control)[a]					Endotoxin (Experimental)[a]				
	34°C		41°C			34°C		41°C		
	Control Glucose, mg/100 ml[b]	60 min Δ Glucose, mg/100 ml	Control Glucose, mg/100 ml[b]	60 min Δ Glucose, mg/100 ml	60 min Q_{10}	Control Glucose, mg/100 ml[b]	60 min Δ Glucose, mg/100 ml	Control Glucose, mg/100 ml[b]	60 min Δ Glucose, mg/100 ml	60 min Q_{10}
Mean(±SE)	95(5)	-14(3)	95(5)	-24(3)	2.78(0.27)	95(5)	-24(3)	95(6)	-35(4)	2.12(0.07)

Comparisons: 0.001^e, 0.005^d, 0.001^c, 0.02^d, 0.05^e

[a] Endotoxin (0.025 mg/ml) or comparable volume of saline (0.025 ml/ml). Absolute concentrations of glucose are shown at control designations.

[b] Absolute concentrations of glucose.

[c] Effect of temperature on glucose uptake.

[d] Effect of endotoxin on glucose uptake.

[e] Effect of endotoxin on metabolic rate (Q_{10}).

TABLE 4. Excess glucose* uptake _in vitro_ in blood after addition of E. coli endotoxin

Number of Experiments	Temperature		Change in glucose concentration from zero time at 60 min (mg/100 ml)		Glucose* Uptake (mg/100 ml)	WBC/mm³ @ 60 min	Mg Glucose Uptake per WBC @ 60 min
			Saline Control	Endotoxin (LD$_{100}$)			
8	34°C	Mean	-14	-24	10	15,300	6.8×10^{-9}+
		SE	3	3	2	2,051	1.3×10^{-9}
8	41°C	Mean	-24	-35	11	15,700	6.7×10^{-9}+
		SE	3	4	3	2,032	1.5×10^{-9}
6	38°C	Mean	-14	-28	14	14,600	9.5×10^{-9}+
		SE	2	4	2	1,835	1.3×10^{-9}

*Calculated excess glucose utilized by 60 minutes (endotoxin minus saline control).   +No significant difference between values at 34°C, 38°C, and 41°C ($p > 0.05$).

TABLE 5. Effects of E. coli endotoxin (LD$_{100}$) on white blood cell concentration in _vitro_*

| | 34°C | | | 38°C | | | 41°C | | |
| | Control (WBC/mm³) | 240 min Δ WBC | | Control (WBC/mm³) | 180 min Δ WBC | | Control (WBC/mm³) | 220 min Δ WBC | |
		Saline	Endotoxin		Saline	Endotoxin		Saline	Endotoxin
Mean (±SE)	16,300(2,035)	-1,325(425)	-2,850(540)	15,300(1,722)	+367(489)	-1,733(809)	16,300(2,035)	-375(367)	-3,025(824)
N	8			6			8		
Paired "t" between changes	NS			0.01			0.02		

*Endotoxin added after transfer of blood to _in vitro_ system.

TABLE 6. Excess glucose* uptake in vitro in blood after E. coli endotoxin addition

Number of Experiments	Temperature		Change in glucose concentration at 60 min (mg/100 ml)		Glucose* Uptake (mg/100 ml)	WBC/mm @ 60 min	Mg Glucose Uptake per WBC @ 60 min
			Saline Control	Endotoxin (LD$_{100}$)			
		Non-activated blood					
6	38°C	Mean	-12	-20	9	11,700	7.2x10^{-9}†
		SE	1	3	2	1,084	1.4x10^{-9}
		Activated blood*					
6	38°C	Mean	-45	-57	12	40,400	4.3x10^{-9}†
		SE	8	5	8	9,634	2.9x10^{-9}

*Calculated excess glucose utilized at 60 minutes (endotoxin minus saline control). †No significant difference (p>0.05). *Blood obtained from animals pretreated with sublethal endotoxin, days 1-3.

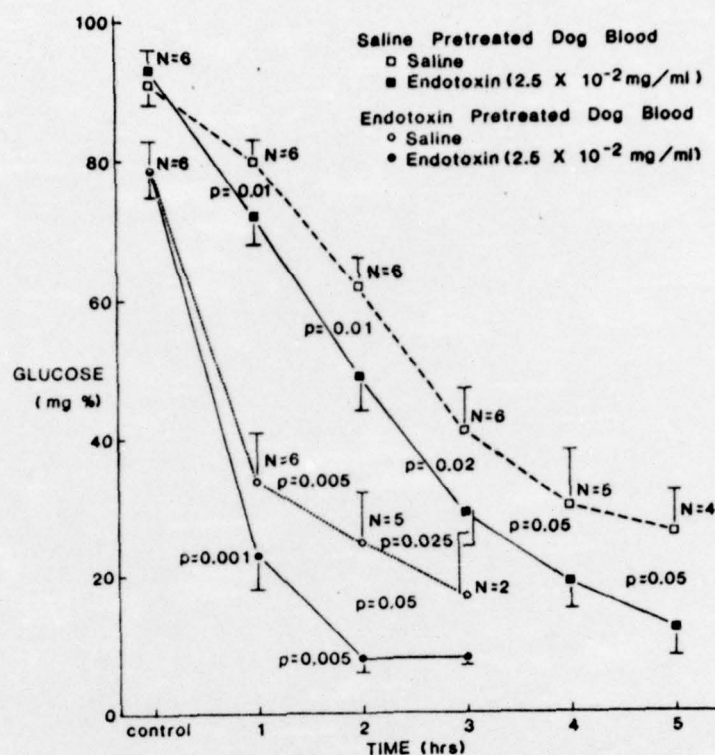Figure 1. Effect of E. coli endotoxin (LD$_{100}$) in blood on glucose uptake in vitro in activated vs. non-activated blood. Activation was produced by prior sublethal injections of endotoxin in vivo (see text), carried out during a 3-day period in unanesthetized dogs (non-activated blood was obtained from animals receiving saline injections instead of endotoxin). Results depict mean in vitro blood glucose concentrations in two groups of 6 blood studies. The lower two curves give values for the in vitro blood given an LD$_{100}$ endotoxin (lower curve) and saline (upper curve) administered to the activated blood while the upper curves provide results in the non-activated blood, following addition of LD$_{100}$ endotoxin or saline.
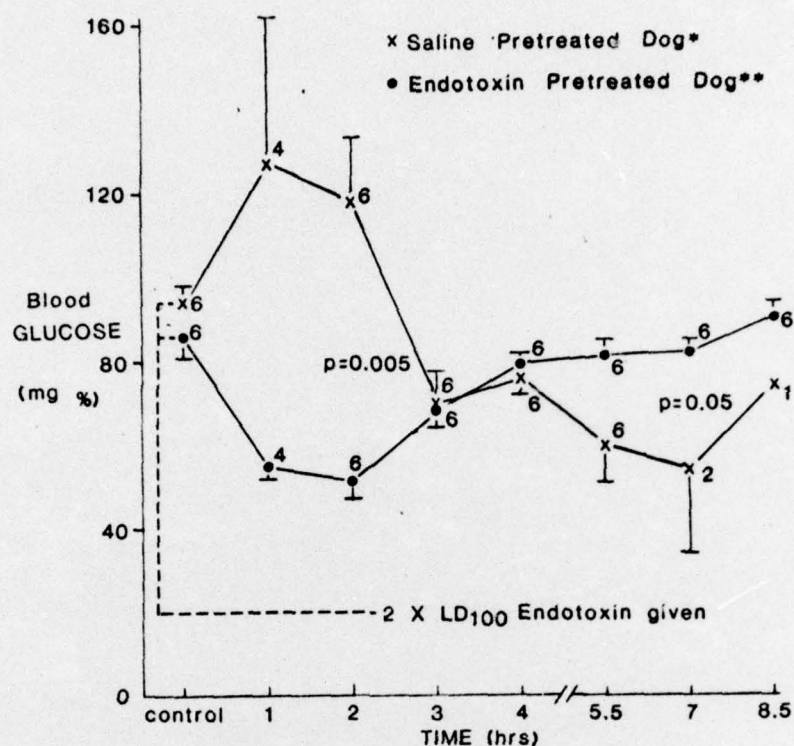
Figure 2A. Effects of intravenous pretreatment with sublethal E. coli endotoxin in vivo on blood glucose concentrations following 2 X LD$_{100}$ E. coli endotoxin. Mean results from 12 dogs are depicted; 6 from group pretreated with sublethal endotoxin (activated)** and 6 control animals receiving saline injections in place of endotoxin (non-activated)*. Both groups received 2 X LD$_{100}$ endotoxin on day of challenge (day 4). Statistical significances between groups are indicated.
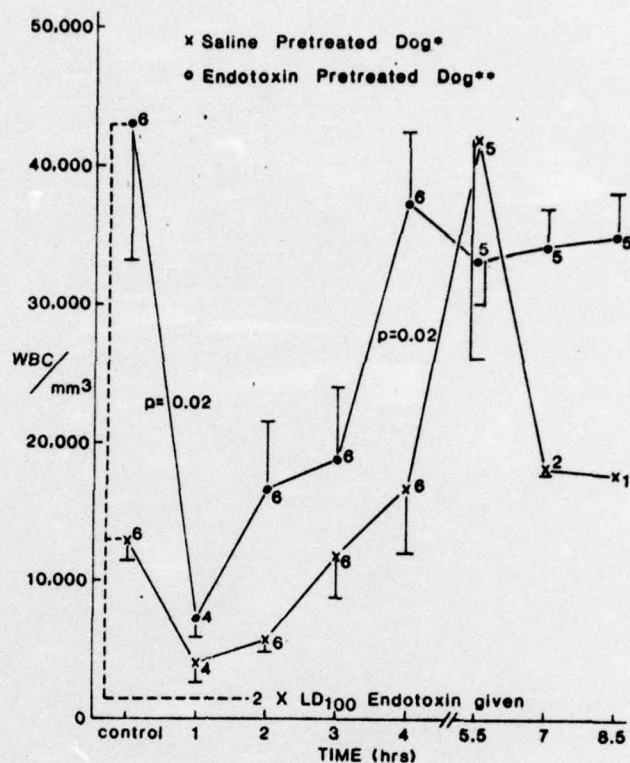
Figure 2B. Effects of intravenous pretreatment with sublethal E. coli endotoxin in vivo on white blood cell concentrations following 2 X LD$_{100}$ E. coli endotoxin. (See Figure 2A for details of experiments.)
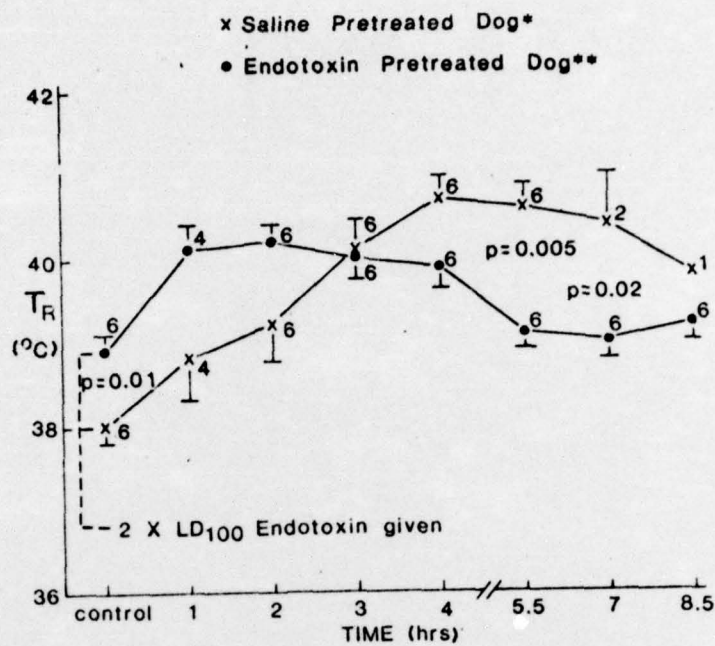
Figure 2C. Effects of intravenous pretreatment with sublethal E. coli endotoxin in vivo on body temperature following 2 X LD$_{100}$ E. coli endotoxin. (See Figure 2A for details of experiments.)

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13. ABSTRACT

The present in vitro study was conducted to explore influences modifying glucose uptake in canine blood administered an estimated LD100 E. coli endotoxin. Particular emphasis was given to assay the role that leukocytes perform in glucose utilization. Results show significant increases in glucose uptake and lactic acid production 1-3 hours following endotoxin addition. Accelerated glucose uptake greatly exceeded basal values and was attributed to increased activity of the white cell. The excess glucose required by the white cell was independent of temperature between the ranges of 34°- 41°C. Endotoxin, however, simultaneously exerted adverse effects by depressing glucose metabolism below predicted values on the basis of Q_{10} findings and increased white cell mortality rate. Blood pretreated with sublethal doses of endotoxin in vivo was studied under in vitro conditions. All endotoxin-pretreated animals survived superlethal doses of endotoxin. Pretreated blood studied in vitro exhibited accelerated hypoglycemia when subjected to endotoxin, "activation" of the blood elicited by increasing numbers of white blood cells rather than by enhancement of individual cell activity.

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